

Table XXI
Study of in vivo Nuclease Resistance of chimeric C3'-endo
(2'-O-MOE) and C2'-endo (2'-ara-OMe) modified
oligonucleotides with and without nuclease resistant caps
(2'-5'-phosphate or phosphorothioate linkage
with 3'-O-MOE in cap ends).

SEQ ID NO:	Sequence	Backbone	Description
30	5'-ATG CAT TCT GCC CCA AGG A-3'	P=S, 2'-H	(control) rodent C-raf antisense oligo
31	AoToGo CoAsTs TsCsTs GsCsCs CsCsAo AoGoGo Aa	P=O/P=S/P=O	2'-MOE/2'-ara-OMe/ 2'-MOE
32	AsTsGs CsAsTs TsCsTs GsCsCs CsCsAs AsGsGs A	P=S	2'-MOE/2'-ara-OMe /2'-MOE
33	Ao*ToGo CoAsTs TsCsTs GsCsCs CsCsAo AoGoGo *A	P=O/P=S/P=O	In asterisk, 2'-5' linkage with 3'-O-MOE;2'-O-MOE/ 2'-ara-OMe/2'-O- MOE/2'-5' linkage with 3'-O-MOE in asterisk;
34	As*TsGs CsAsTs TsCsTs GsCsCs CsCsAs AsGsGs *A	P=S	In asterisk, 2'-5' linkage with 3'-O-MOE;2'-O-MOE/ 2'-ara-OMe/2'-O- MOE/2'-5' linkage with 3'-O-MOE in asterisk.

PROCEDURE 8

Animal studies for in vivo nuclease resistance

[0288] For each oligonucleotide to be studied, 9 male BALB/c mice (Charles River,

Wilmington, MA), weighing about 25 g are used (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277, 923). Following a 1-week acclimation, the mice receive a single tail vein injection of oligonucleotide (5 mg/kg) administered in phosphate buffered saline (PBS), pH 7.0. The final concentration of oligonucleotide in the dosing solution is (5 mg/kg) for the PBS formulations. One retro-orbital bleed (either 0.25, 9.05, 2 or 4 post dose) and a terminal bleed (either 1, 3, 8 or 24 h post dose) is collected from each group. The terminal bleed (approximately 0.6-0.8 mL) is collected by cardiac puncture following ketamine/xylazine anesthesia. The blood is transferred to an EDTA-coated collection tube and centrifuged to obtain plasma. At termination, the liver and kidneys will be collected from each mouse. Plasma and tissues homogenates will be used for analysis for determination of intact oligonucleotide content by CGE. All samples are immediately frozen on dry ice after collection and stored at -80 °C until analysis.

PROCEDURE 9

RNase H studies with chimeric C3'-endo and C2'-endo modified oligonucleotides with and without nuclease resistant caps

³²P Labeling of Oligonucleotides

[0289] The oligoribonucleotide (sense strand) was 5'-end labeled with ³²P using [³²P]ATP, T4 polynucleotide kinase, and standard procedures (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., in *Current Protocols in Molecular Biology*, John Wiley, New York (1989)). The labeled RNA was purified by electrophoresis on 12% denaturing PAGE (Sambrook, J., Frisch, E. F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Plainview (1989)). The specific activity of the labeled oligonucleotide was approximately 6000 cpm/fmol.

Determination of RNase H Cleavage Patterns

[0290] Hybridization reactions were prepared in 120 µL of reaction buffer [20 mM Tris-HC (pH 7.5), 20 mM KCl, 10 mM MgCl₂, 0.1 mM DTT] containing 750 nM antisense oligonucleotide, 500 nM sense oligoribonucleotide, and 100,000 cpm ³²P-labeled sense

oligoribonucleotide. Reactions were heated at 90 °C for 5 min and 1 unit of Inhibit-ACE was added. Samples were incubated overnight at 37 °C degrees. Hybridization reactions were incubated at 37 °C with 1.5×10^{-8} mg of *E. coli* RNase H enzyme for initial rate determinations and then quenched at specific time points. Samples were analyzed by trichloroacetic acid (TCA) assay or by denaturing polyacrylamide gel electrophoresis as previously described [Crooke, S. T., Lemonidis, K. M., Neilson, L., Griffey, R., Lesnik, E. A., and Monia, B. P., Kinetic characteristics of Escherichia coli RNase H1: cleavage of various antisense oligonucleotide-RNA duplexes, *Biochem J*, 312, 599 (1995); Lima, W.F. and Crooke, S. T., *Biochemistry* 36, 390-398, 1997].

[0291] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is therefore intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.